The use of *Anacardium occidentale* as nutraceutical in hypoprotein diets for laying hens

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One hundred sixty White Leghorn hens (Hybrid L-33) of 30 weeks of age in their full laying peak were utilized for 91 d to assess the use of *Anacardium occidentale* as nutraceutical in hypoprotein diets for laying hens. A completely randomized design, with four treatments of foliage and sprouts powder of *Anacardium occidentale* (0; 0.5; 1.0 and 1.5%) were applied as nutraceutical additive. There was no morbidity or mortality with the inclusion of this powder in the diets. The addition of up to 1.5% of *A. occidentale* powder increased the production (75.50 to 80.00%), egg weight (61.20 to 62.37 g), the height of the dense white (6.72 to 7.78 mm), the thickness (0.36 to 0.41 mm) and eggshell surface (75.90 to 78.24 cm^2). The intensity of the yolk's color (10) was marked by the addition of 0.5% of powder. The addition of this medicinal powder did not decrease the sensory albumen quality and the egg yolk. There were no alterations in the hematological examination to the hens at 30 and 43 weeks. However, hypoprotein diets affected total plasma proteins (44.70 to 47.00 g/L). The use of 1.5% of foliage and sprouts powder of *Anacardium occidentale* is recommended for hypoprotein diets for laying hens for increasing egg production and quality, without affecting the sensory quality or the health indicators of the birds.

Key words: *Anacardium occidentale*, *quality, hypoprotein, laying hen, production*

The constant antibiotic application as additives for animal feeding could provoke an increase of the number of resistant strains, besides transferring crossed resistance to other microorganisms. Preventive medicine insists more and more to limit the utilization of antibiotics as growth or production promoters (AGP). Despite these contraindications, many development countries continue to use them widely (Fuente *et al*. 2009).

Natural products constitute an alternative in front of the abuse of antibiotics. In poultry industry the properties of many functional or nutraceutical feeds as the prebiotics, probiotics, enriched and plant extracts, for improving health condition, decrease the pathogen microorganisms and modulate a better immunity response (Gong *et al*. 2002) have been studied.

From the technical, economical and biological points of view, plant additives are considered an alternative to substitute antibiotics, in view of the security of their inclusion and their almost null residual effect (Kong *et al*. 2004, Kong *et al*. 2007 and Ayala *et al*. 2011). The future premise of the researchers is to find natural alternatives to counteract the indiscriminate use of antibiotics as preventive in birds. Some benefits have been reported from the powder of medicinal plants, related to the increase in nutrient digestibility, immunological stability, competitive exclusion of microorganisms and intestinal health in birds apparently normal and exposed to different stress conditions (Kong *et al*. 2007, Fuente *et al*. 2009 and Martínez *et al*. 2011).

Anacardium occidentale is a tree native to Brazil, that can be localized any place. Shows diverse medicinal properties, as hypoglycemic and antihypertensive activity in rats, anti-mollusk against slugs (*Biomphalaria glabrata*) and possess bactericide, vermifuge and anti-inflammatory (Sokeng *et al*. 2001) action. Foliage and sprouts powder has been employed effectively to combat the diarrhea syndrome in poultry, pigs, guinea pigs, sheep, rabbits, bovines and humans (Martínez and Martínez 2012). Also, its addition in diets of chick replacers of laying hens increases the productive indicators, reduces serum glucose and the intestinal hypersensitivity (Gallardo 2011 and Martínez *et al*. 2011). Despite all the medicinal benefits of the leaves and sprouts powder of *A. occidentale* there is not enough data on its application as additive in poultry diets. The objective of this study was to evaluate de use of *Anacardium occidentale* as nutraceutical in hypoprotein diets for laying hens.

Materials and Methods

Location of the experimental area. The experiment was carried out in the Poultry Unit "San Andrés" situated at the outskirts of the municipality of Holguín, belonging to the Eastern region of Cuba. Mean relative

humidity was 67% and average minimum and maximum temperatures were 17 and 25.9ºC, respectively (data from the Meteorological Station of Holgín).

Preparation of A. occidentale powder and phytochemical screening. Twenty kg of leaves and sprouts from three *A. occidentale* trees were collected from the surrounding urban areas of Holguín. These territories are characterized by a flat topography and red ferrallytic soil. In the collection the size and the structure of the leaves was considered.

The product was dehydrated at environmental temperature for 72 h and later shredded in a hammer mill with parallel blades at 1 mm granule size. Samples were stored at environmental temperature in hermetically sealed plastic bags (Yin *et al*. 1993).

Phytochemical screening was carried out to ethanolic, aqueous and ether extracts obtained from foliage and sprouts of *A. occidentale*. For this, 10 g of sample were weighed in a technical balance with precision ± 0.10 g (BS 2202S SARTORIUS, Germany) and successive extractions with increasing polarity solvents (petroleum ether, alcohol and water), were made.

As measurement criterion the cross system was used to quantify the secondary metabolites (table 1). The phytochemical screenings were made according to the methodology described by Miranda and Cuellar (2000) at the Centro de Inmunología y Biopreparados de Holguín, Cuba.

Animals and treatments. One hundred sixty White Leghorn (Hybrid L-33) laying hens were distributed for 13 weeks according to a completely randomized design with four treatments, five replications per treatment and eight birds per replication.

Hypoprotein diets were prepared with the addition of 0; 0.5; 1.0 and 1.5 % of *A. occidentale* foliage and sprouts powder in the first laying phase. Diets were based on wheat and soybean cake, according to the recommendations of UECAN (2011). Therapeutic results and chemical composition of *A. occidentale* powder were considered for the selection of the nutraceutical addition (Martínez *et al*. 2011 and Martínez and Martínez 2012). Table 2 shows the contribution of the chemical composition calculated from the experimental diets for laying hens.

Experimental conditions. The experimental unit was a 60 x 80 cm metallic cage, where 8 hens were assigned. Birds received 108 g of feed/hen/d. Water was supplied *ad libitum* by three nipple drinkers per cage. Sixteen hours of illumination were provided daily. There was no adaptation period to the experiment (Kong *et al*. 2004). No medicine or therapeutic veterinary care was offered during the experimental stage.

Productive indicators. Initial and final weighing of the laying hens was individually performed at 30 and 43 weeks of age, through a digital SARTORIUS (model BL 1500) balance, with precision ± 0.10 g. Egg weight was taken twice weekly to 30 eggs/treatment, between 8:30 and 9.30 a.m. and average weight was estimated. *A. occidentale* and total tannin consumption were determined daily by the offer and rejection method. Total tannin concentration in *A. occidentale* powder (22.73 mg/g) (Martínez and Martínez 2012) was taken into account.

For determining laying intensity, total egg production/week/treatment was considered. One egg/d/bird housed was assumed as 100%. Mass conversion was calculated through feed consumed, egg weight per replication and number of eggs laid. At the end of the experiment, the viability was also calculated.

Percentage of eggs not fit for consumption (cracked,

Table 1. Phytochemical screening of the *A. occidentale* foliage and sprouts powder

Secondary metabolites	Ether extract	Ethanolic extract	Aqueous extract
Sudan III (fatty acids)	+		
Mayer and Wagner (alkaloids)	-	+	+
Baljet (coumarines)	+	++	
Foam (saponins)		+	+
Shinoda (flavonoids)		+	+
Essential oils	+		
Wagner (alkaloids)		+	
Mucilage			-
Fehling (reducing sugars)		+	-
Bortrager (quinones)		-	+
Libermamn-Burchard (triterpenes and/or steroids)		+	
Ninhidrina (free amino acids)		+	
Anthocyanidines		+	
Ferric chloride (phenols and/or tannins)		+ (Pirocatecolics)	-

- Absence
+Presence
+Abundant

Table 2. Calculated chemical composition of the *A. occidentale* powder diets for laying hens (30 to 43 weeks)

Treatments	CP (%)	GF (%)	CF (%)	Ca (%)	P (%)
Control	15.50	1.67	2.40	3.80	0.66
0.5 %	15.56	1.70	2.50	3.80	0.66
1.0 %	15.62	1.72	2.61	3.80	0.66
1.5 %	15.68	1.74	2.71	3.81	0.66

CP – crude protein
GF – gross fat
CF – crude fiber

without an eggshell and broken) was calculated by the formula: % of eggs not fit for consumption (ENFC) = #ENFC* 100/eggs fit for consumption.

External and internal egg quality indicators. At 43 weeks of the experiment, 30 eggs/treatment were sampled to determine the external quality indicators (weight, thickness, eggshell surface) and internal quality indicators (height of the dense white and of the yolk, Haugh units and yolk color). A Russian slide gauge with ± 0.01 mm precision was used for measuring the eggshell thickness at the egg's equator and at the upper and lower poles. The shell surface was established according to Carter's (1975) formula:

Area = 3.9782P0.7056, where P is the egg weight (g)

The height of the dense white and of the yolk was measured with a height gauge with ± 0.01 mm accuracy (Maver). The records of Haugh units were calculated by the relationship between the egg weight and the height of the dense white:

HU = 100 log (H + 7.75 – 1.5W037), where:

HU are the Haugh units

H is the height of the white

W is the egg weight

The yolk color was determined by Roche's range of 15 colors.

Sensory quality. For the sensory yolk and albumen evaluation, eggs were tempered in water bath at 50ºC for 3.25 min., without salt. The assessment panel was integrated by ten tasters, between 20 and 55 years of age, enjoying excellent health. The sensory quality criteria tested were: smell (pleasant, rancid and others), taste (normal, rancid and odd) and texture (soft or fibrous), according to Schreiner *et al*. (2004).

Hematological examination. At 43 weeks of the experiment, a hematological examination was realized to five fasting hens/treatment. Blood was extracted by a puncture to the vein of the left wing, using insulin (1 mL) syringes and later placed in 2 mL flasks with sodium heparin. Leucocites were analyzed by blood smear and Giemsa dye; hemoglobin, by the method Hemotest; hematocrit according to Wintrobe and total proteins by Biuret, read by a spectrophotometer Shimadzu. The hematological analyses were carried out at the Immunology Laboratory attached to the University of Medical Sciences (Holguín), certified by the Ministry of Public Health (MINSAP).

Statistical analysis. Data were processed by analysis of variance (Anova) of simple classification, in completely randomized design. In the necessary cases Duncan's (1955) test was applied to determine mean differences, according to the statistical software SPSS version 12.1. The percentage of cracked eggs and without an eggshell was analyzed by comparison of proportions in the statistical software COMPARPRO 1.0 (Font *et al*. 2007).

Results and Discussion

Table 3 shows the productive indicators of laying hens, fed different addition levels of *A. occidentale* powder. Viability did not show significant differences between treatments, demonstrating the innocuousness of the product used for 91 d. These results coincide with Martínez *et al*. (2011), who did not find morbidity-lethality in replacement chicks of laying hens. These findings are similar to those obtained by Martínez and Martínez (2012) when laboratory mice were fed to determine chronic toxicity for 3 months. Worku *et al*. (2009) and Ayala *et al*. (2011) reported the effectiveness of medicinal plants due to their low residual effect, when applied as nutraceutical in the animals.

The 4.50 % increase in the laying intensity, with the addition of 1.5% of *A. occidentale* powder demonstrated the effectiveness of this medicinal plant as stimulating in diets of laying hens. This suggests its utilization when birds are exposed to nutritional stress, specially, of protein type. Ghasemi *et al*. (2010) found similar responses on adding garlic powder (*Allium sativum*) and thyme (*Thymus vulgaris*) in laying hens diets, which confirms the beneficial effect of natural products, as *A. occidentale* powder.

According to Gimeno (2004) and Ghasemi *et al*. (2010), the secondary metabolites as the phenolic compounds found in the *A. occidentale* powder (table 1), could be phytoestrogenic. This could have influenced on the increase of egg production and decrease of mass conversion of the birds fed with this medicinal powder (table 3). According to Grobas *et al*. (1999), the estrogen increase in blood stimulates egg production in laying hens.

In table 3 is shown the increase in total tannin intake

Table 3. Effect of *A. occidentale* powder on the productive performance of laying hens (30 to 43 weeks)

Indicators	Addition of *A. occidentale* powder (%)				SE± Sig.
	0	0.50	1.00	1.50	
Viability (%)	100.00	100.00	100.00	100.00	
Laying intensity (%)	75.50[c]	75.90[c]	77.60[b]	80.00[a]	1.09**
Feed intake (g/bird/d)	108.00	108.00	108.00	108.00	
A. occidentale intake (g/bird/d)	0.00	0.54	1.08	1.62	
Tannin intake (g/bird/d)	0.23	0.24	0.25	0.27	
Mass conversion (kg/kg)	2.34[a]	2.32[a]	2.24[b]	2.16[c]	0.09**
Egg weight (g)	61.20	61.00	61.88	62.37	0.40
Initial live weight (g)	1600.00	1615.00	1590.00	1598.00	20.47
Final live weight (g)	1637.00	1640.00	1639.00	1642.70	22.98
Cracked eggs (%)	0.33	0.22	0.00	0.00	0.12
Broken eggs (%)	0.00	0.00	0.00	0.00	
Eggs without an eggshell (%)	0.11	0.11	0.00	0.00	0.07

[a,b,c]Means with different letters in the same row differ at $P < 0.05$ (Duncan 1955) **$P < 0.01$

(0.04 g/bird/d), due to *A. occidentale* intake. Although tannins are considered anti-nutritional factors, when used in small concentrations in the diets constitute efficient bactericides, fungicides, antioxidants, mineral chelates and astringents (Gimeno 2004, Sousa de Brito and Pessanha de Arau 2007 and Martínez *et al*. 2011). Thus, the inclusion of these beneficial polyphenols in small amounts in bird diets, are recommended.

The coumarines (++) and reducing sugars, found by phytochemical screening (table 1) and by thin layer chromatography in the *A. occidentale* powder (Gallardo 2011), have beneficial effects in small proportions and are strong anticoagulants and bactericides against *Staphylococcus aureus* and *Escherichia coli* strains (Martínez and Martínez 2012). Vieira *et al*. (2006) reported in *A. occidentale* bactericide activity in face Streptococcus species, indicating that *A. occidentale* powder can contribute to the competitive exclusion of the microorganisms in the gastrointestinal tract, since birds suffering nutritional stress are more susceptible due to the possible increase of the pathogen bacteria population latent in this portion. In view of the valuable characteristics of *A. occidentale*, it can be stated the direct effect of the secondary metabolites of the birds, taking into account that these are not synthetized by them.

In this study, feed intake did not decrease in spite of the greater *A. occidentale* and total tannins consumption. These results do not agree with those reported by Martínez *et al*. (2011), who with the addition of 1.5 % of *A. occidentale* powder found consumption decrease in young birds. This suggests that birds with better body and digestive development can assimilate greater concentrations of secondary metabolites (Gallardo 2011).

Live weight of laying hens did not show significant differences between treatments, though the secondary metabolites added in the diets did not provoke symptoms related to anti-nutritional factors, but according to Savón *et al*. (2007), too much of these decrease body weight.

Egg weight throughout all the experimental weeks did not decrease with the inclusion of this medicinal powder. Faria *et al*. (2003) indicated that methionine is the most important essential amino acid for egg weight. This suggests that total tannin intake was not excessive, since tannins can prevent the absorption of sulfureous amino acids (Savón *et al*. 2007). According to data shown in table 3, the addition of this powder in the feed did not affect the percentage of eggs fit for consumption.

In table 4 can be seen that the addition of *A. occidentale* powder in the diets increased egg weight, eggshell thickness and superficial area at week 43. The possible increase of estrogens in blood could have influenced on these results. According to Elaroussi *et al*. (1994), estrogens are the main calcic carbonate carriers from intestine to bones and, in turn, enter to the calcium homeostatic cycle. This makes feasible to avoid calcium unbalance in bones, as well as the incidence of problems in the legs of the animals and in the thickness of the eggshell. A probable improve in intestinal health of the birds with *A. occidentale* could have influenced on calcium digestion and absorption, since according to Savón *et al*. (2007) proper pH conditions are required due to the insolubility or instability of this mineral.

The addition of *A. occidentale* powder increased the height of the dense white in correspondence with the egg weight. This confirms that total tannin consumption did not decrease the absorption of sulfureous amino acids. According to Keener *et al*. (2006), the white amount depends on the amino acid balance contributed by the protein of the diet. A lysine or methionine deficiency reduces the albumen weight and decreases the concentration of all the free amino acids. Likewise, the height increase of the dense white with *A. occidentale*

Table 4. Effect of the *A. occidentale* powder on the external and internal quality of the egg of laying hens (43 weeks)

Indicators	Addition of *A. occidentale* powder (%)				SE± Sig.
	0	0.5	1.0	1.5	
Egg weight (g)	64.30[b]	66.55[ab]	65.60[ab]	68.16[a]	0.87*
Eggshell thickness (mm)	0.36[c]	0.39[ab]	0.37b[c]	0.41[a]	1.75**
Eggshell surface (cm^2)	75.90[b]	76.92[ab]	76.16[ab]	78.24[a]	0.70*
Height of the dense white (mm)	6.72[b]	7.17[ab]	7.14[ab]	7.78[a]	0.33**
Yolk height (mm)	13.92[b]	14.70[ab]	14.86[ab]	15.34[a]	0.33***
Haugh units	79.89[b]	82.42[ab]	81.96[ab]	86.44[a]	1.50*
Yolk color	8.00[c]	10.00[a]	9.00[b]	9.00[b]	0.09**

[a,b,c]Means with different letters in the same row differ at $P < 0.05$ (Duncan 1955) *$P < 0.05$ **$P < 0.01$ ***$P < 0.001$

powder influenced on the Haugh units, as egg quality indicator (table 4).

The yolk color (table 4) was marked with the addition of *A. occidentale* powder. According to the phytochemical screening (table 1), this medicinal plant contains anthocyanidines, a natural dye that according to Carrillo *et al*. (2005), influences on yolk pigmentation. Nonetheless, this result indicates that faster egg formation could determine pigment deposition in the yolk, as it occurred in treatments with 1 and 1.5 % of *A. occidentale* powder where there was a color weakening regarding 0.5 % of *A. occidentale* powder (table 4).

The egg sensory quality of laying hens (data not shown) was not affected by the addition of *A. occidentale* powder. Secondary metabolites in this medicinal powder did not provoke unpleasant savors.

Results from the hematological examination of laying hens (table 5) did not show statistical differences. This demonstrates the effectiveness of this medicinal powder, provoking no hematological alterations and not diminishing the defenses (white globules). However, Ghasemi *et al*. (2010) on utilizing two medicinal powders in diets for laying hens found 8.16 % increase in the lymphocites.

The hemoglobin value (table 5) indicates that the addition of *A. occidentale* powder could have not affected iron absorption. According to Gimeno (2004) and Martínez *et al.* (2011), tannins prevent the absorption of this mineral, which can provoke anemia due to iron deprivation. Also, the low concentration of protein in

Table 5. Effect of the *A. occidentale* powder on the hematological examination of laying hens

Indicators	Weeks	Addition of *A. occidentale* powder (%)				SE±
		0	0.5	1.0	1.5	
Leukocytes ($x10^9$/L)	30	20.50	22.25	26.50	20.00	2.55
	43	21.75	20.50	21.75	20.75	1.93
Polymorphous ($x10^9$/L)	30	0.28	0.23	0.18	0.23	0.04
	43	0.26	0.27	0.26	0.26	0.003
Lymphocytes ($x10^9$/L)	30	0.70	0.72	0.79	0.73	0.05
	43	0.72	0.71	0.72	0.71	0.003
Monocites ($x10^9$/L)	30	0.05	0.20	0.25	0.15	0.10
	43	0.01	0.02	0.03	0.02	0.005
Eosinofiles ($x10^9$/L)	30	0.00	0.00	0.00	0.00	
	43	0.00	0.00	0.00	0.00	
Myelocites ($x10^9$/L)	30	0.00	0.00	0.00	0.00	
	43	0.00	0.00	0.00	0.00	
Hemoglobin (g/L)	30	91.00	88.50	89.50	87.00	2.58
	43	89.00	88.75	88.25	87.25	1.40
Hematocrit (u/L)	30	0.29	0.30	0.27	0.27	0.02
	43	0.29	0.28	0.27	0.28	0.01
MCCH (g/L)	30	313.75	295.30	331.00	322.75	13.56
	43	307.25	317.50	326.50	311.75	8.43
Plasmatic proteins (g/L)	30	45.25	44.50	45.75	47.25	2.78
	43	44.75	45.50	47.00	44.70	3.26

MCCH . Mean concentration of corpuscular hemoglobin

the experimental diets decreased the circulation of this biomolecule in the hens' blood. According to Causey (2000), the normal values of the White Leghorn hens range in 54.5 b/L, explaining that all birds were below the reference range.

The use of 1.5 % powder of foliage and sprouts of Anacordium occidentale in hypoproteic diets for laying hens is recommended for increasing egg production and quality, without affecting its sensory quality or the health indicators of the birds.

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